ELSEVIER

Journal of Chromatography A, 960 (2002) 175–185

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Position and intensity of system (eigen) peaks in capillary zone electrophoresis ☆

H. Sellmeyer, H. Poppe*

Research Group on Polymer Analysis, Institute of Technical Chemistry, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

Abstract

The intensity of system (or eigen) peaks encountered in capillary zone electrophoresis (CZE) can be predicted by considering mass balances for each of the analyte constituents and each of the constituents in the background electrolyte (BGE). As a result of coherence, in each zone the proportions in which the constituent concentrations vary are fixed; they are determined by the composition of the BGE and the nature of the analyte constituent (if present) and described as eigenvectors of a transport matrix. Considering the effect of an injection, the mass balances for all constituents can be satisfied only via the intensity of each zone. This leads to an *n*-equations, *n*-unknowns problem, with the intensities as the unknowns and the mass balances as equations.The latter can be easily solved to obtain the intensities of the zones, of analytes as well as of system peaks. In this work the approach has been applied to CZE systems with two co-ions in the BGE, and experimental results have been compared to the predictions obtained from the model. Agreement was seen to be reasonable, but the quantitative comparison often failed, probably due to experimental difficulties.

Keywords: System eigenpeaks; Ghost peaks; Indirect detection; Eigen peaks

1. Introduction

System (or ‘‘ghost-’’ or ‘‘eigen-’’) zones in capillary zone electrophoresis (CZE) have been studied by many workers [1–14]. These zones move through the medium as if they are analyte zones, but consist only of disturbances in the concentrations of the background electrolyte (BGE) and contain no analytes. They may or may not be detectable, depending on whether the BGE contains a detector-responsive constituent. Even if this is not the case, they may cause baseline fluctuations via refractive index effects. These studies have been mainly done in the context of indirect detection, where indeed the existence of these zones can very much deteriorate the reliability of CZE analysis, by the appearance of disturbing peaks in the electropherogram. Even when this does not occur, because the system zones are ‘‘invisible’’, they may very well disrupt the peak shapes of analytes sitting at the same position [3,7]. From most of these papers a important message emerges: the simpler the composition of the BGE, the fewer system zones are present, and the smaller is the risk for such undesired complications. However, in some cases it is favourable, for other

☆Presented at the International Symposium on Column Liquid Chromatography and Related Methods, ‘‘HPLC-Kyoto’’, Kyoto, 11–14 September 2001.

*Corresponding author. Tel.: +31-20-636-6844; fax: +31-20-525-5604.

E-mail address: h.poppe@chello.nl (H. Poppe).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00242-X

reasons, to use a slightly more complicated BGE. E.g., the requirement on the monitoring ion in indirect detection, together with that on adequate, pH-buffering, the requirements for regulation of electroosmotic flow, and the need to add anti-oxidants may lead to such situations.

The cited papers contain valuable practical rules for the prediction of system peak positions for simple BGE systems, which can be described adequately by using the Kohlrausch regulating function (KRF) [15]. Also, more sophisticated mathematical tools have been applied [4,7,8] to handle more complicated situations, e.g., polybasic buffers.

However, it remained unclear how the intensities of system zones are related to the injected solution, and to the properties of the BGE. In two previous papers [16,17] we have indicated how such relations can be found in principle. This treatment was based on the formulation of the relevant electromigration transport equations under conditions of minor disturbance in terms of a linear matrix formulation, leading to an eigenvector problem [16–19]. The minor disturbance assumptions allow one to linearize the problem. In this way very complicated and time consuming in-detail calculations and simulations [20–23], that often do not give much general insight, can be avoided. In this contribution the matrix approach is worked out further, and some experimental results are compared to the predictions following from our model.

2. Theory

In our approach constituents rather than ions are used as the basic variables. A constituent is defined as a component irrespective of its degree of protonation. Thus H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} are all constituent ''phosphate''. Giving the concentrations of all such constituents defines a solution unambiguously via the pK_a values and the required electroneutrality; e.g., 50 millimolar ''acetate constituent'' and 25 millimolar sodium (ion) represent a BGE buffer with nearly equal concentrations of acetic acid and sodium acetate, with a pH close to the pK_a of acetic acid.

The dimensionality of the system, n, is equal to the number of constituents, i.e., n numbers describe the composition.

If only salts of strong ions occur in BGE and sample, it is nearly always fairly accurate to neglect the influence of H^+ and OH^- (pH assumed to be around 7); in that case one needs one number less to describe the solution; e.g., in mixed KCl and NaCl, c_{Na} and c_K would suffice to define the solution as c_{Cl} is fixed by the electroneutrality condition. The dimensionality, n, is in that case equal to the number of different ions minus 1.

It is assumed throughout this paper that all concentrations stay close to that in the BGE (minor disturbances). Each concentration will often be described by its relative value, Δc, indicating the difference with the concentration in the BGE. Δc values can be negative, except for analytes. A composition can be described by n numbers, giving the Δc_i values for all constituents (with the BGE concentrations as origin). These n values can be taken as a vector, $\mathbf{\Delta c}$.

As has been put forward in a number of papers [18,24,19,17,16], for conditions of minor disturbance a most general description of the coupled migration of analyte and BGE constituents is obtained when the coupled differential transport equations for all constituents are forced into a matrix form, an idea originally proposed (for chromatography) by Mangelsdorf Jr. [25] and Riedo and Kovats [26], for gas chromatography (GC) and liquid chromatography (LC), respectively, and e.g., used in high-performance liquid chromatography (HPLC) [27,28]. The eigenvalues of this matrix correspond to the velocities (or, when suitably scaled, mobilities) of the zones. The eigenvectors describe the proportions between Δc values in one zone. Thus, in a zone the $\mathbf{\Delta c}$ vector is equal to an eigenvector, apart from a multiplicative constant. The constant is related to the intensity, I, of a zone. The zones satisfy the coherence condition as formulated (be it for chromatography, but see Ref. [17]) by Helffrich and Klein [29,30].

The matrix elements are found from:

$$A_{i,j} = I_e/F \left[\delta(i,j)\,{}^a\mu_i/\kappa + c_i \frac{\partial({}^a\mu_i/\kappa)}{\partial c_j} \right] \qquad (1)$$

where I_e is the electrical current density, E is the electric field strength, F is Faraday's constant, $\delta(i,j)$ is the Kronecker Delta, 1 if $i=j$, 0 if $i<>j$, ${}^a\mu_i$ is the average or effective mobility of constituent i, c_i and

c_j are the concentrations of constituents i and j, respectively. κ is the electrical conductivity:

$$\kappa = I_e/E = F\frac{\sum_{\text{all ions } k} E\mu_k z_k s_k}{E} = F\sum_{\text{all ions } k}\mu_k z_k s_k \qquad (2)$$

where s_k and μ_k values are the concentrations and mobilities of the individual ions (e.g., $H_2PO_4^{2-}$), respectively, and where H^+ and OH^- should also be counted.

In a few, very simple, cases it is worthwhile to derive analytical expressions for the $A_{i,j}$. However, in general, in a program developed by us, the values of the $A_{i,j}$ are found by numerical differentiation of expressions like Eq. (1). The steps involved in this rather complicated process are described in Ref. [17].

For a sample ion s, with zero concentration in the BGE, the second term in Eq. (1) vanishes; the elements $A_{s<>k}$ are all zero, while the $A_{s,s}$ element equals the average velocity, [a]u_i. Thus, the latter value is a eigenvalue for each sample ion.

For the very simplest capillary electrophoresis (CE) experiment, e.g., injection of sodium chloride into a BGE of potassium chloride, these ideas can be illustrated as follows: the dimensionality is 2 (pH 7, no effect of H^+ or OH^-, chloride follows from electroneutrality, in BGE as well as in the zones).

One eigenvalue (''1'') is the mobility of sodium. For this zone the $\mathbf{\Delta c}$ values for sodium and potassium can be calculated to be (see below) in the proportion $0.626/(-0.727)$. This says that where sodium is present, potassium is lower than in the BGE. Later on in the text this will be formulated as: the corresponding vector is ($\mathbf{e}_{1,\mathrm{Na}} = 0.626$, $\mathbf{e}_{1,\mathrm{K}} = -0.727$). This also explains why the two numbers 0.626 and -0.727 are given, rather than just their ratio; the eigenvectors are usually given such that their euclidic length is 1.

The other eigenvalue (''2'') for this situation is zero, the corresponding vector is ($\mathbf{e}_{2,\mathrm{Na}} = 0$, $\mathbf{e}_{2,\mathrm{K}} = 1$). The zone consists of slightly diluted or slightly concentrated BGE, containing no sodium. Such a zone with zero mobility is also present in more complicated BGEs and is indicated in the following as the ''EO-zone'', as its presence often allows to determine the electroosmotic flow mobility.

Once the $n*n$ matrix $\mathbf{A}$ consisting of the elements $A_{i,j}$ has been found, its eigenvalues λ_k and eigenvectors $\mathbf{e_k}$ can be found with a numerical package. The λ_k correspond to the velocities of allowed (coherent) zones. When emerging from Eq. (1), they would be in terms of velocity, so a division by the field strength is needed to convert them into zone mobilities, μ_k. It follows from the theory of eigensystems that there are n λ_k values with corresponding $\mathbf{e_k}$ values.

As coherence is the ''natural'' [30] end state to which a system evolves, any disturbance that has a different character (i.e., does not have the proportions in Δc_i values prescribed by one of the $\mathbf{e_k}$ vectors) will eventually be split in two or more zones that do have such proportions.

This then leads the way to an understanding of the relation between the injected sample solution (amount and composition) on the one hand and the intensity of all zones on the other hand by means of an integral mass balance for every constituent.

The relative concentration of a constituent i in a resolved zone k can be written as:

$$\Delta c_{i,k} = e_{i,k} G_k(z - u_k t) \qquad (3)$$

where $G_k(z-u_k t)$ describes the intensity and shape of the zone. The shape may reflect the original injection profile, or may be distorted more or less, by dispersion or electromigration dispersion, or by isotachophoretic effects occurring in the first stages after injection. In Eq. (3) u_k is the velocity of zone k, $\mu_k E$, z is the longitudinal position in the capillary and t is the time.

Note that an eigenvector is only determined in its direction, the values of its components can be multiplied by an arbitrary constant without impairing its validity. Usually, and also here, the vectors are normalized such that their euclidic length is 1, i.e., the sum of squares of the components is 1. Note also that the G-function bears no index i, indicating that the shape of all Δc_i curves versus t or z are the same, their height proportions being given by the $e_{k,i}$ values.

The relative amount contained in one zone k, for constituent i, $\Delta W_{i,k}$ is equal to:

$$\Delta W_{i,k} = A_c e_{i,k} \int_{-\infty}^{-\infty} G(z - u_k t)\mathrm{d}z \qquad (4)$$

where A_c is the cross-sectional area of the capillary.

Eq. (4) can be written as:

$$\Delta W_{i,k} = e_{i,k} I_k \tag{5}$$

In Eq. (5) I_k represents the integral of $G(z-u_k t)$ in Eq. (4) times A_c. Apart from a correction factor amounting to u_k (cf. Ref. [31]), and the sensitivity of the detector, it is equal to the integrated signal observed with common signal integrators. I_k values are identified as the intensities of zones.

From the integral mass balances for all constituents the intensity of all zones can be found: When an injection of length l_S has been made, with concentration vector $\mathbf{\Delta c_S}$, the total amount in the capillary of a constituent i differs from the background value by:

$$\Delta S_i = A_c l_S \Delta c_{i,S} \tag{6}$$

where S and index S stands for sample (injection).

After the injection plug has been fully resolved in zones, but before any zone has left the capillary, this difference must still be the same, as there no net loss of gain of material has occurred. Thus for each constituent i it must hold:

$$\Delta S_i = \sum_{\text{all zones } k} \Delta W_{i,k} = \sum_{\text{all zones } k} I_k e_{i,k} \tag{7}$$

The ΔS_i are known from the injection conditions; the $e_{i,k}$ can be found from the properties of the BGE and the analytes by means of the eigenvalue/vector calculation. A set of equations such as Eq. (7), valid for each of the n constituents i, is an example of n equations with n unknowns, I_k, the $e_{i,k}$ being the coefficients and the S_i being the constant terms. This set of equations can be solved by standard linear algebra; inversion of the matrix **E**, consisting of the $e_{i,k}$ elements, gives the desired result:

$$\mathbf{I} = (\mathbf{E})^{-1}\mathbf{\Delta S} \tag{8}$$

where **I** is the vector of length n of intensities I_k, $\mathbf{\Delta S}$ is the vector of length n of injection amounts, ΔS_i.

The process is graphically illustrated in Fig. 1, with 0.01 mol/l imidazolium chloride as the BGE and 0.005 mol/l sodium chloride as the sample. Imidazolium is treated here as a strong ion, so that the pH is assumed to be 7; the system, BGE plus sample, has dimensionality 2 and can be represented in a plane. The two possible eigenvectors in $\{e_{\text{Imidazole}}, e_{\text{Na}}\}$ are {0.707, 0.707} with $\mu = 0$ (electroosmotic marker zone, short EO-zone) on the one hand and $\{-0.698, 0.716\}$ with $\mu = 48$ $(= \mu_{\text{Na}})$ on the other hand. Note that, as mentioned before, the absolute value of the vector components can be

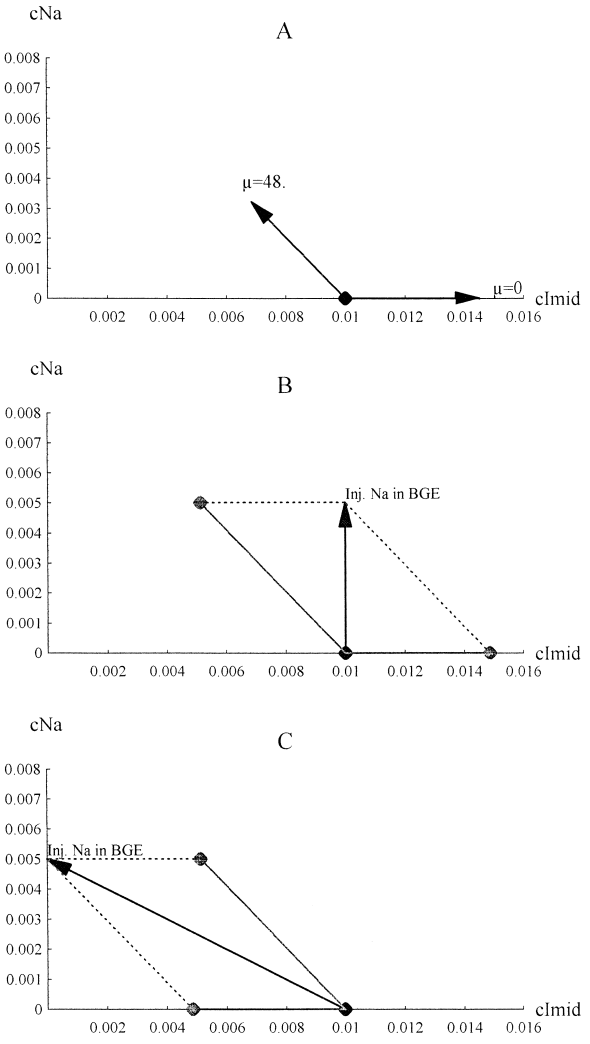

Fig. 1. Two-dimensional vector representation of decomposition of injection vector into eigenvectors of coherent zones. BGE consists of imidazolium chloride with sodium chloride as a sample. Dimensionality of combination (BGE+Na) is 2 (imidazole treated as strong ion in this figure). (A) The two (eigen)vectors allowed by the coherence condition, specified only in direction, not yet in length. Mobility of corresponding zone indicated. (B) Injection with Na dissolved in BGE, grey lines ending in filled circles represent intensity of zones, direction same as vectors in A. (C) Same as B, except for injection with Na dissolved in water.

chosen arbitrarily, only their ratios matter. Therefore, $\{e_{\text{Imidazole}}, e_{\text{Na}}\}=\{0.707, 0.707\}$ just means that the two concentrations vary in the same proportion, the value 0.707 is in fact $1/\sqrt{2}$, chosen to make the euclidic length $\sqrt{(e^2_{\text{Imidazole}}+e^2_{\text{Na}})}$ equal to one, as required by convention. However, in Fig. 1A (and Fig. 2A) this convention has not been used (in fact this would be without meaning, as the scales in these figures have the unit mol/l, while the eigenvector components have no dimension). The graphical length of the eigenvectors has been chosen to fit the figure; their direction is the only thing that matters.

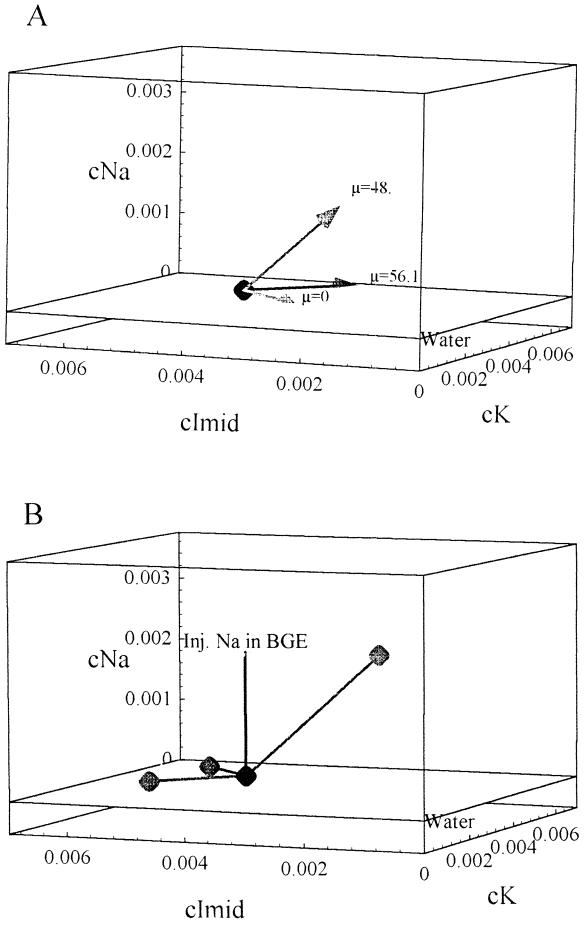

Fig. 2. Three-dimensional representation of the decomposition into eigenvectors for a BGE consisting of 0.005 mol/l imidazolium chloride and 0.005 mol/l potassium chloride with sodium chloride as a sample. Dimensionality of combination (BGE+Na) is 3 (imidazole treated as strong ion in this figure). (A) The three (eigen)vectors allowed by the coherence condition, specified only in direction, not yet in length. Slope of Na-vector ($dc_{\text{Imid}}/dc_{\text{Na}}$) equals 1/(transfer ratio) [32]. (B) Injection with Na dissolved in BGE, grey lines ending in filled circles represent intensity of zones, direction same as vectors in A.

The injection vector for an injection and its decomposition has been drawn for two cases: sodium chloride dissolved in water (Fig. 1B) and dissolved in BGE (Fig. 1C). This is the elementary case occurring in a basic treatment of indirect detection [32,10]: the slope of the Na-vector equals 1/(transfer ratio) [32], and is here equal to $-0.698/0.716=-0.974$. It should be noted that such a simple case can be readily analyzed without resort to the numerical method used here, e.g., by using the KRF [33–35,12,2].

The logic behind the present treatment can be made clearer by considering the difference in response in the EO-zone in Fig. 1B and C. In Fig. 1C, with injection from water, the EO-zone is strongly negative, due to the deficit in imidazole brought about by the injection. In Fig. 1B, however, the EO-zone is positive. Since in the latter case the total amount of imidazole has not been changed by the injection, the negative content (relative to BGE) in the Na-zone must be compensated somewhere in the system by a positive deviation. As the EO-peak is the only place where a deviation from the BGE composition occurs, this compensation should be there. This reasoning is in fact what Eq. (7) says.

A three-dimensional representation for a system with three constituents is given in Fig. 2. The BGE is assumed to consist of potassium and imidazolium (again treated as a strong ion) chloride, while sodium is injected. As the BGE itself now has two degrees of freedom, there are two system zones, one with mobility zero, and one with mobility in between that of potassiun and imidazolium. For the first the eigenvector points to the ''water point'', as $e_{\text{K}}=e_{\text{Imidazolium}}$. For the second, the eigenvector components e_{K} and $e_{\text{Imidazolium}}$ have opposite signs; the zone can be described as an exchange between potassium and imidazolium. The analyte vector, protruding out of the shaded ''BGE-plane'', has non-zero components in both potassium and imidazole.

When dealing with an injection with many constituents, a simplification can be obtained as follows: when the BGE has p constituents, and the injected solution contains many, say q, other constituents, it is not needed to carry out the eigenvector calculation

and the inversion Eq. (7) for the full matrices of dimension $n=p+q$. As the response to the complex injection is built up as a linear combination of the responses to each of the analytes, each analyte injected can be treated separately; each time matrices of dimension $p+1$ have to be handled. The responses in the q analyte zones are totally independent (for analytes not occurring in the BGE one can identify a zone with the analyte as constituent, as its zone is the only one in which its concentration varies). For the q system zones the intensities are found by adding up the intensities brought about by each of the q analytes.

3. Experimental

A Prince CE instrument (LauerLabs Emmen, The Netherlands) was used in combination with a UV detector type SpectroFlow 757 (ABI, Ramsey, NJ, USA), used at 206 nm (maximum of imidazole absorption). The capillary was 79 cm (44.5 cm to the detector window)×375 μm O.D.×50 μm I.D. All chemicals used were at least analytical grade. Pretreatment of the capillary involved washing with 0.1 mol/l sodium hydroxide for 5 min, then water for 2 min and then BGE for 10 min. Injection was done hydrodynamically; running voltage was 25 kV. Experiments were carried out at room temperature, which varied between 17 and 22 °C.

Electropherograms were recorded with a recorder type BD 111 (Kipp&Zn, Delft, The Netherlands), and for obtaining peak integrals also recorded on a personal computer (PC) 486 DX66 with appropriate interface card and the electrophoresis data processing software CAESAR 3.0 (LauerLabs).

Calculations were carried out on the PC, with a program NPEL.EXE, custom written in Turbo Pascal, version 7.0 for DOS (Borland International, Scotts Valley, CA, USA). Algorithms for eigenvector/value calculations and matrix inversion were taken from Ref. [36].

Mobilities and pK_a values, needed for the program, were taken from the compilation by Hirokawa [37], except for the mobilities of analytes and co-ions (all cations), which were determined beforehand in a buffer of suitable pH and the same ionic strength as in the experiments.

Table 1
Mobilities used in the calculations

Ion	μ ($\cdot 10^{-9}$ m^2/V/s)	pK_a (from Ref. [37])
Na^+	48.0	
Li^+	38.0	
K^+	67.0	
Imidazolium$^+$	46.0	6.8
Ephedrinium$^+$	25.0	9.9
From literature		
Cl^-	−79	

Measured, at 18–20 °C, ionic strength 0.1 mol/l, pH 4.3.

4. Results and discussion

4.1. Imidazole BGE system

Table 1 list the values for mobilities and pK_a values for the constituents used in the software. In the following text mobilities will be indicated by their values in the unit $\cdot 10^{-9}$ m^2/s/V.

Table 2 pertains to an experiment where 0.01 mol/l neat imidazolium chloride was used as the BGE. This is a simple system, that could have been calculated by using the KRF, except that in our numerical approach the small effects of the acid–base reaction of Imidazole and the contribution of hydrogen ion to the conductivity is taken into account. The pH was measured as 4.44, which agrees reasonably with the value of 4.40 given by the program. Injected was a mixture of potassium, lithium and sodium chloride, each 0.001 mol/l, dissolved in either water or BGE.

In all experiments the peak areas were compared to the calculated predictions in relative terms. That is, every peak area, experimental or predicted, was divided by that of a suitable reference peak, in this case that of the sample constituent sodium. In this way painstaking experiments for determination of the absolute sensitivity of detector and integrator were avoided.

The calculated values in Table 2 for the peak area ratios are not the same for the four BGE experiments. This stems from the influence of the electroosmotic flow on the peak area (cf. Ref. [31]). The electroosmotic flow was not stable between experiments.

The precision of our area measurements was in the

Table 2
Peak area ratios, experimental vs. calculated

Injection in	K/Na		Li/Na		SysEO/Na	
	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated
BGE	0.67	0.66	1.02	0.93	−4.79	−5.7
BGE	0.67	0.68	0.99	0.93	−5.30	−5.2
BGE	0.65	0.69	0.88	0.91	−2.48	−4.5
BGE	0.67	0.70	0.85	0.91	−2.93	−4.4
Water	0.5	0.66	0.67	0.82	34.5	16.5

BGE: 0.01 mol/l, imidazolium$^+$ Cl^-. Injected 0.001 mol/l Na, 0.001 mol/l K and 0.0007 mol/l Li.

range of 5% relative standard deviation. Thus the last three data on SysEO/Na are definitely out of the expected range. We have no explanation for that, except that the electroosmotic flow may have varied even during the run. Nevertheless, Table 2 is satisfactory in that the model predicts the sign as well as the order of magnitude of the system peak response correctly.

4.2. Imidazole/potassium BGE system

Results obtained with more complicated BGE systems are reported in Figs. 3–5. The BGE always contained 0.01 mol/l chloride, and varying proportions of potassium (0.2, 0.6, 0.8 and 0.9 times the chloride concentration) and so much imidazole that the pH was 6.8. Injected was a mixture of sodium and lithium chloride, 0.001 mol/l each, dissolved in either water or the BGE, as indicated in the figures.

In Fig. 3 the comparison between observed and predicted mobilities of the system peak with mobility in between that of imidazole and that of potassium (''SysIII''; name does not have any special significance) is visualized. Both are plotted as a function of the fraction of potassium chloride in the total ionic strength [thus c_{K^+}/c_{Cl^-}, the imidazole total concentration is than about $2\cdot(c_{K^+}-c_{Cl^-})$, for obtaining pH 6.8]. As can be seen, the agreement is again reasonable, although quantitatively not very precise.

In Fig. 4 the comparison between observed and predicted peak area ratios is shown for the injection with the sample dissolved in water, whereas in Fig. 5 this is done for sample dissolved in BGE. In both cases the sodium peak was taken as the reference.

In both Fig. 3 on the one hand, and Figs. 4 and 5 on the other hand the agreement appears to be better with BGE as the injection solvent than when water is used as the injection solvent. Possibly this is again related to the disturbance of the electroosmotic flow velocity within the run, which can be expected to be larger when water is injected.

4.3. Ephedrine/potassium BGE system

This system was chosen because it allows to study the peak sign inversions when a system peak is either on the one, or on the other side of the analyte peak in the electropherogram. This situation was not reached with imidazole/potassium, where the mobility of the system peak (''SysIII'') is, for practical fractions of potassium, always larger than those of the two analytes, lithium ($\mu=38$) and sodium ($\mu=48$). With the mobility of ephedrine ($\mu=25$), a system peak (''SysIII'') can be expected to lie somewhere between $\mu=68$ (potassium), and $\mu=25$ depending on the composition. Thus when varying the fraction potassium, the system peak (''SysIII'') will ''move'' subsequently through both analyte peaks. As has been discussed by others and us [9,3,4,16], such a situation gives rise to (i) peak sign inversion for indirect detection and when analyte and system peak have nearly the same mobility, (ii) excessive response factors and (iii) excessive peak asymmetry. In this work we were interested in the peak sign inversions and the behaviour of system peak (''SysIII'').

The various BGEs were prepared in an analogous was as in the imidazole/potassium experiments. Thus, chloride concentrations were maintained at 0.01 mol/l; potassium concentrations were chosen to be 0.2, 0.4, 0.6, 0.8 and 0.9 times this value, and ephedrine was added to reach a pH of 9.9, for which about $2\cdot(c_{K^+}-c_{Cl^-})$ was needed. Injection was

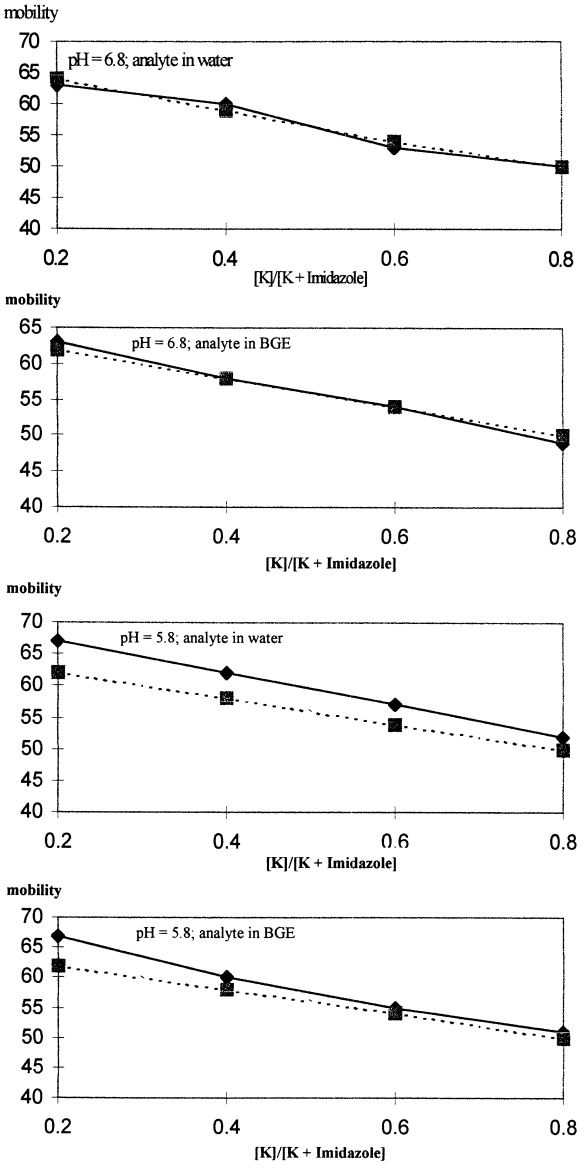

Fig. 3. Mobility of system peak II (exchange of K^+ and imidazolium$^+$) experimental (full line) and calculated (dotted line). BGE: Mixture of imidazolium chloride and potassium chloride, with varying K^+/imidazolium$^+$ ratio, with fixed ionic strength 0.01 mol/l at pH 6.8 ($=pK_a$). Horizontal axis: fraction $c_{K^+}/(c_{K^+}+c_{Imid})$. Injected: NaCl, 0.001 mol/l, dissolved as indicated.

analogous to the imidazole/potassium case, again 0.001 mol/l for each of the analytes.

Fig. 6 shows the observed and predicted values for the mobility of the system peak (''SysIII'') as a

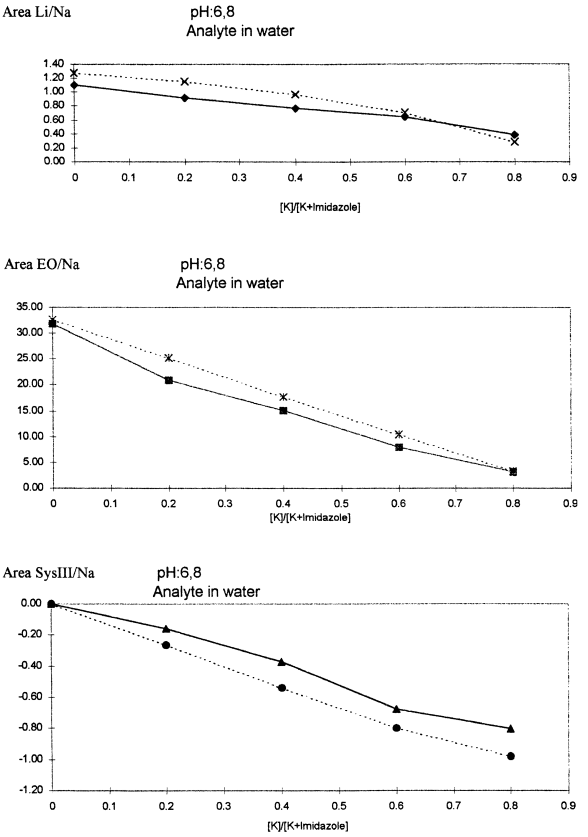

Fig. 4. Peak area ratios, experimental (full line) and calculated (dotted line). BGE: Mixture of imidazolium chloride and potassium chloride, with varying $c_{K^+}/c_{Imidazole^+}$ ratio, with fixed ionic strength 0.01 mol/l at pH 6.8. Horizontal axis: fraction $c_{K^+}/(c_{K^+}+c_{Imidazole^+})$. Injected: NaCl 0.001 mol/l and LiCl 0.001 mol/l, dissolved in water.

function of the potassium fraction. The impression is the same as for Fig. 4: the trend is predicted well, but quantitative agreement is not quite satisfactory. Still, we believe that the model producing the predictions is reliable, and that experimental artifacts are behind these deviations.

In Fig. 7 the comparison for the peak areas are made, for injection from water. For this set of experiments we decided to use the ''EO-peak'' as the reference peak, as this is the only one that does not change sign when the fraction f_K is varied. The format of the figure is different from that of Figs. 4 and 5; the predicted values are shown as lines rather than as points. This was done because the predicted points were quite off-scale for the values 0.4 and 0.6

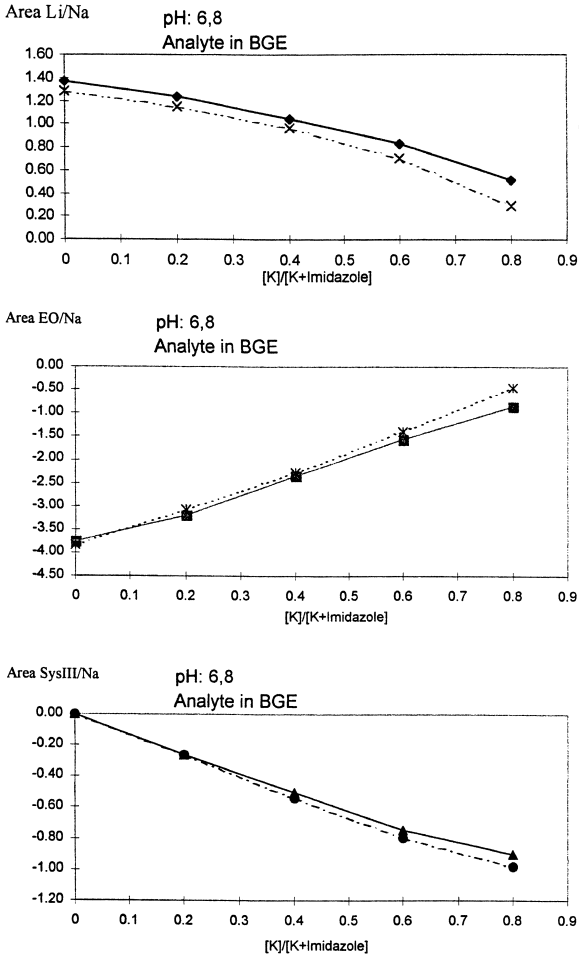

Fig. 5. Peak area ratios, experimental (full line) and calculated (dotted line). BGE: Mixture of imidazolium chloride and potassium chloride with varying $c_{K^+}/c_{Imidazole^+}$ ratio, with fixed ionic strength 0.01 mol/l at pH 6.8. Horizontal axis: fraction $c_{K^+}/(c_{K^+}+c_{Imidazole^+})$. Injected: NaCl 0.001 mol/l and LiCl 0.001 mol/l, dissolved in BGE.

of the horizontal axis; apparently at these values the system peak nearly coincided, in the calculation, with the peaks of sodium and lithium, respectively. This is also visible in Fig. 7. We have to assume that in the actual experiments the separation between system peak (''SysIII'') and the analytes peaks was always larger, so that no such excessive responses were obtained. In retrospect it would have been better to choose the potassium fraction in the BGE more judiciously.

Fig. 8 shows the same data, but now for an

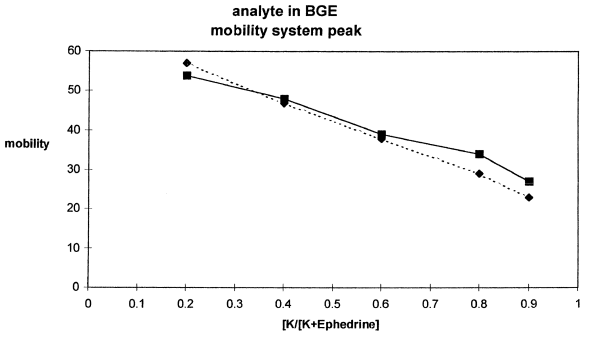

Fig. 6. Mobility of system peak ''SysIII'' (exchange of K^+ and ephedrinium$^+$) experimental (full line) and calculated (dotted line). BGE: Mixture of ephedrinium chloride and potassium chloride, with varying $c_{K^+}/c_{Ephedrinium^+}$ ratio, with fixed ionic strength 0.01 mol/l at pH 9.9. Horizontal axis: fraction $c_{K^+}/(c_{K^+}+c_{Ephedrine^+})$. Injected: NaCl, and LiCl each 0.001 mol/l, dissolved in BGE.

injection from water, of lithium and sodium, 0.001 mol/l each.

Figs. 7 and 8 demonstrate that the predictions are qualitatively (i.e., sign of response, and large response factors for some cases) in agreement with the experimental results. However, the quantitative agreement is unsatisfactory, if not poor. Several explanations can be given: again, variation in EO-flow may have played a role. In Fig. 7, the intensity of the EO-peak, used as a reference, was very large, and possibly the integrated areas were affected by detector non-linearity. Finally, it was observed several times during this and other work at high pH that carbon dioxide is absorbed pretty fast in such BGE stock solutions. This could have lead to significant shifts in pH and BGE properties. One reviewer made the remark that also other contaminants, especially those with a mobility close to that of the relevant zones, may lead to quite unexpected results.

At least, this experiment shows that the use of BGEs with more than one co-ion must be avoided at any price, as it leads to very unpredictable results, a fact noted before by numerous workers [9,3,35,11].

5. Conclusion

A framework for obtaining insight into the intensity of system peaks in CZE has been developed. The

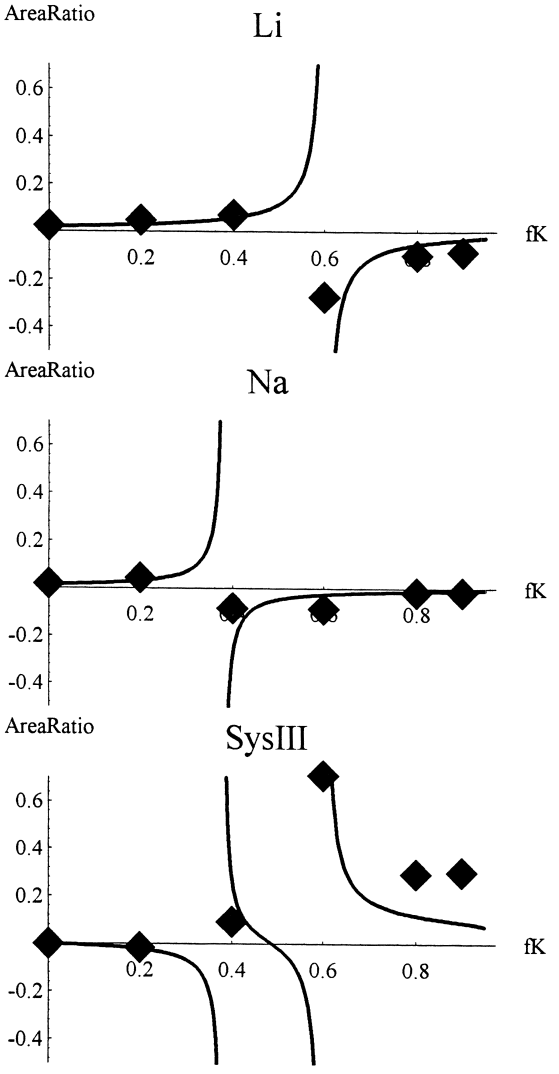

Fig. 7. Peak area ratios, experimental (filled diamonds) and calculated (line). BGE: Mixture of ephedrinium chloride and potassium chloride, with varying $c_{K^+}/c_{Ephedrinium^+}$ ratio, with fixed ionic strength 0.01 mol/l at pH 9.9. Horizontal axis: fraction $c_{K^+}/(c_{K^+}+c_{Ephedrine^+})$. Injected: NaCl and LiCl 0.001 mol/l, each 0.001 mol/l, dissolved in water.

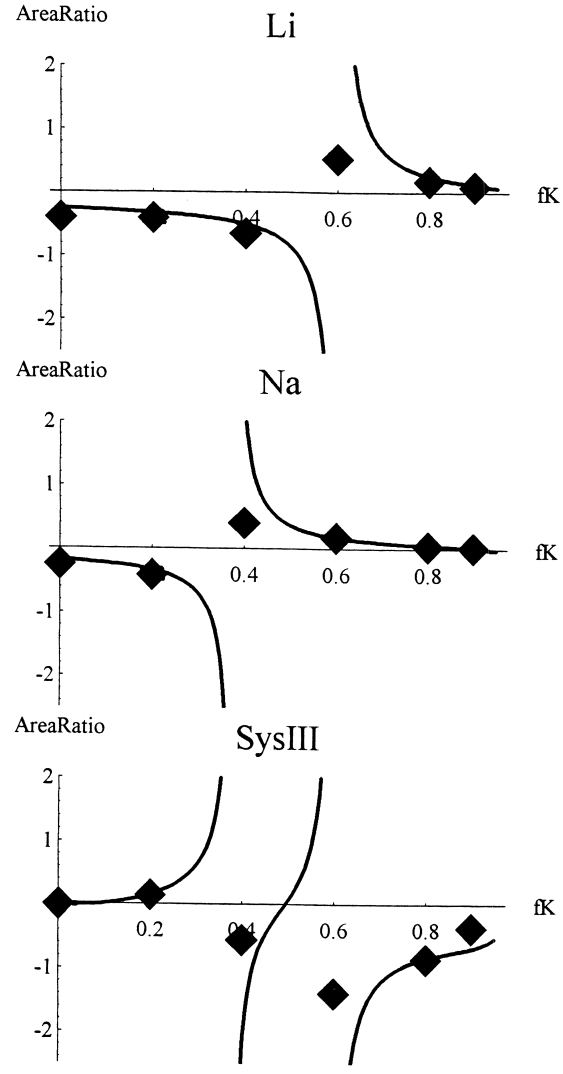

Fig. 8. Peak area ratios, experimental (filled diamonds) and calculated (line). BGE: Mixture of ephedrinium chloride and potassium chloride, with varying $c_{K^+}/c_{Ephedrinium^+}$ ratio, with fixed ionic strength 0.01 mol/l at pH 9.9. Horizontal axis: fraction $c_{K^+}/(c_{K^+}+c_{Ephedrine^+})$. Injected: NaCl and LiCl 0.001 mol/l, each 0.001 mol/l, dissolved in BGE.

approach has been successful also for the purpose of studying peak deformations due to electromigration dispersion [38,39]. The experimental verification presented here on system peak intensities is not quite satisfactory, at least in terms of quantitative agreement. However, as there is, as far as we can see, no reason to question the underlying mathematical treatment, we believe that the framework is basically correct. There is one exception to that: The indirect detection response is calculated in the program as it was used is derived from the Δc_m values for the constituent that serves as the ''monitoring ion'' (imidazole and ephedrine in our two sets of experiments . However, the UV-absorption response of

such constituents depend on their acid–base equilibrium. It is known [20,21,40] that within zones the pH can shift from the BGE value. As a result, the UV response in especially relatively concentrated zones may be different form that in more diluted zones. Taking this numerically into account is no problem, but knowledge of the ratio of UV responses for the various protonation forms of the monitoring constituent would be required.

References

[1] C. Desiderio, S. Fanali, P. Gebauer, P. Bocek, J. Chromatogr. A 772 (1997) 81.
[2] P. Gebauer, P. Bocek, J. Chromatogr. A 772 (1997) 73.
[3] P. Gebauer, P. Borecka, P. Bocek, Anal. Chem. 70 (1998) 3397.
[4] J. Beckers, P. Bocek, Electrophoresis 20 (1999) 518.
[5] P. Gebauer, P. Pantuckova, P. Bocek, Anal. Chem. 71 (1999) 3374.
[6] P. Gebauer, P. Pantuckova, P. Bocek, J. Chromatogr. A 894 (2000) 89.
[7] J.L. Beckers, P. Gebauer, P. Bocek, J. Chromatogr. A 916 (2001) 41.
[8] P. Bocek, P. Gebauer, J.L. Beckers, Electrphoresis 22 (2001) 1106.
[9] M. Macka, P.R. Haddad, P. Gebauer, P. Bocek, Electrophoresis 18 (1998) 1998.
[10] P. Doble, M. Macka, P.R. Haddad, Electrophoresis 19 (1998) 2257.
[11] P. Doble, P.R. Haddad, Anal. Chem. 71 (1999) 15.
[12] M.C. Breadmore, P.R. Haddad, J.S. Fritz, Electrophoresis 21 (2000) 3181.
[13] M.C. Breadmore, P.R. Haddad, J.S. Fritz, J. Chromatogr. A 920 (2001) 31.
[14] X. Xu, W.Th. Kok, H. Poppe, J. Chromatogr. A 786 (1997) 333.
[15] F. Kohlrausch, Ann. Phys. 62 (1897) 209.
[16] H. Poppe, in: P.R. Brown, E. Grushka (Eds.), Advances in Chromatography, Marcel Dekker, New York, 1998.
[17] H. Poppe, J. Chromatogr. A 831 (1999) 105.
[18] H. Poppe, Anal. Chem. 64 (1992) 1908.
[19] G.J. Bruin, A.C. van Asten, X. Xu, H. Poppe, J. Chromatogr. 608 (1992) 97.
[20] J.L. Beckers, J. Chromatogr. A 693 (1995) 347.
[21] J.L. Beckers, J. Chromatogr. A 696 (1995) 285.
[22] R.A. Mosher, D.A. Saville, W. Thormann, The Dynamics of Electrophoresis, VCH, Weinhein, New York, Basel, Cambridge, 1992.
[23] R.L. Williams, B. Childs, E.V. Dose, G. Guiochon, G. Vigh, J. Chromatogr. A 781 (1997) 107.
[24] H. Poppe, J. Chromatogr. 556 (1991) 95.
[25] P.C. Mangelsdorf Jr., Anal. Chem. 38 (1966) 1540.
[26] F. Riedo, E.Sz. Kovats, J. Chromatogr. 239 (1982) 1.
[27] J. Crommen, J. Schill, P. Herne, Chromatographia 25 (1988) 397.
[28] J. Crommen, G. Schill, D. Westerlund, L. Hackzell, Chromatographia 24 (1987) 252.
[29] F.G. Helfferich, Ind. Eng. Chem. (Fundam.) 6 (1967) 362.
[30] F.G. Helfferich, G. Klein, Multicomponent Chromatography, A Theory of Interference, Marcel Dekker, New York, 1970.
[31] J.H. van der Moolen, H.C. Smit, H. Poppe, J. Chromatogr. A 744 (1996) 103.
[32] T.W. Garner, E.S. Yeung, J. Chromatogr. 515 (1990) 639.
[33] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[34] B. Gas, J. Vacik, I. Zelensky, J. Chromatogr. 545 (1991) 225.
[35] B. Gas, P. Coufal, M. Jaros, J. Muzikar, I. Jelinek I, J. Chromatogr. A 905 (2001) 269.
[36] W.H. Press, S.A. Teukolsky, W.T. Vetterling, B.P. Flannery, Numerical Recipes, The Art of Scientific Computing, 2nd ed., Cambridge University Press, 1992.
[37] J. Hirokawa, J. Chromatogr. 271 (1983) D1.
[38] A. Cifuentes, H. Poppe, J. Chromatogr. A 680 (1994) 321.
[39] A. Cifuentes, H. Poppe, Electrophoresis 16 (1995) 516.
[40] X. Xu, W.Th. Kok, H. Poppe, J. Chromatogr. A 742 (1996) 211.